

Chapter 18 Recombinant DNA and Biotechnology

1. What is a clone, and what is meant by gene cloning?

A clone is an identical copy, e.g. of a gene. Gene cloning means to make many copies of a given gene by introducing it into a host cell, so that the gene is multiplied together with the host cell. (p389 18.2)

2. Many restriction enzymes recognise palindromic sequences in DNA. Give an example of a DNA palindrome of 6 nucleotides

(p387-388)

For example: GGGCCC, ATATAT, GACGTC, CCGCGG, AGTACT

3. What is the enzyme DNA ligase used for, in the context of gene cloning?

DNA ligase joins two DNA fragments that have complementary “sticky ends”, by making a covalent bond between them. It can also join “blunt ends” together, but this is less effective. (p387-388)

4. Give named examples of cloning vectors and mention their most important features

Plasmid:

It is small

It can replicate independently of the host cell due to the origin of replication

It usually contains a single recognition site for a given restriction enzyme

It usually contains a gene encoding an antibiotic resistance that can be used to select for host cells that have taken up the plasmid

There is a limit to the size of fragment that can be inserted into the plasmid: usually < 10000 basepairs. This means that plasmids are often not suitable for cloning of eukaryotic genes.

Virus:

Larger DNA fragments can be inserted than into plasmids

Natural infection of host cells

Ti-plasmid:

*Can be used for insertion of DNA fragments into many plant species. Encodes genes that allow the bacterium *Agrobacterium tumefaciens* to infect plant cells and insert DNA into the plant genome. Ti plasmid also contains recognition sites for restriction enzymes that can be used to insert fragments.*

(p389-390)

5. How can PCR be used in gene cloning?

If the sequence of a gene is known, specific primers can be designed to amplify the gene from very few copies of a chromosome, and insert it into a cloning vector.

PCR can also be used to amplify DNA after reverse transcriptase has made a cDNA copy of an mRNA.

PCR can be used analytically to screen a number of clones for the presence of a desired DNA sequence

(p393)

6. Explain what is meant by (i) selection (ii) screening

- (i) Functions like natural selection, by ensuring that only cells containing the desired gene can survive. For example antibiotic resistance can be used for selection: by growing bacteria on media containing an antibiotic, only the cells containing a resistance gene can grow.*
- (ii) Searching for an individual with particular properties among many survivors. For example by using a reporter gene like green fluorescent protein, that fluoresces under UV light. Antibiotics can also be used for screening: the text book contains an example where a plasmid carries two different antibiotic resistance genes – one is used for selection and the other for screening.*

(p390-391)

7. What is needed to ensure that a gene introduced via a plasmid is expressed in a host cell?

The plasmid must have an origin of replication. The gene must have a promotor and transcriptional terminator that are functional in the host organism, as well as signals for start and stop of translation (start and stop codons). It is also possible to optimize the codon usage of the gene to match the host organism.

(p397-398)

8. What is a genomic library?

e.g. a bacterial culture where each recombinant cell contains a fragment of a given genome. Together, they represent the genome.

(p392, Fig 18.6)

9. What is cDNA?

Complementary DNA (cDNA) is a DNA copy of an mRNA, made by the enzyme reverse transcriptase
(p392)

10. What is a gene-chip (DNA array) and what can it be used for?

It consists of thousands of different known DNA sequences, each at a precise location on the chip. Often a chip will contain all the open reading frames or genes from a whole genome. These can be used to identify the genes that are expressed in a specific tissue at a certain time. This is done by

probing each of the single stranded DNA sequences on the chip with mRNA or cDNA isolated from the tissue (and labelled with a fluorescent probe), to see at which specific positions on the chip hybridization occurs.
(fig. 18.9 p396)

11. How is gene technology different from traditional plant breeding methods?

*Genes can be transferred between distantly related or unrelated species
The genetic modification is known at the DNA level.
Traditional breeding takes much longer to obtain a uniform variety with properties that are stable over many generations*

(p400)

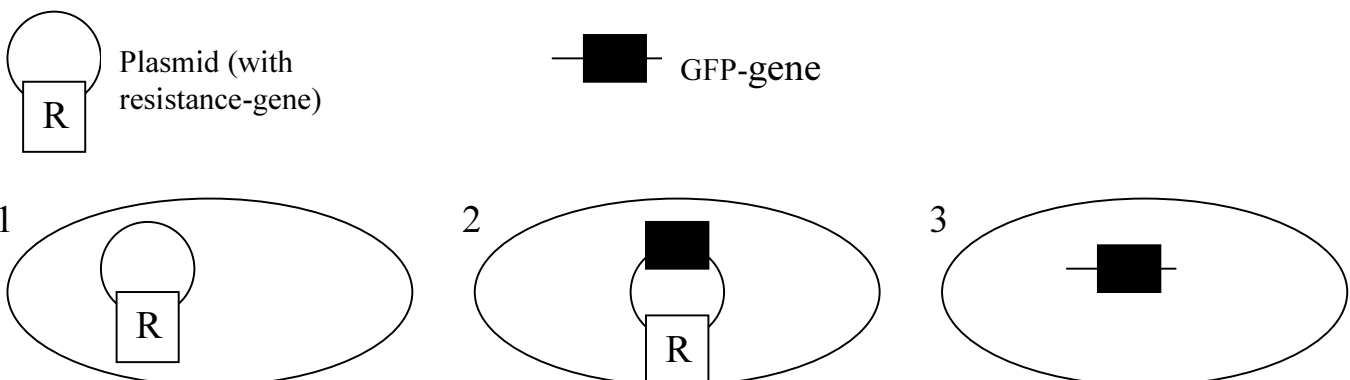
12. What is your opinion about the practical applications of gene technology- what is acceptable and what should be forbidden?

13. What are transgenic animals, and in which context can they be used?

Transgenic animals contain modified DNA, genes have been added or removed. They can be used to investigate the function of a gene, for example by studying the effect of deactivating the gene. They can be used to produce medicines, for example production of growth hormone in cow's milk.

(p. 399-400)

14. You carry out an experiment in which you insert a DNA fragment encoding GFP (Green fluorescent protein, a protein that fluoresces under UV light) into an expression vector (plasmid) and transfer it to bacteria. The plasmid already contains a gene that encodes an antibiotic resistance gene. Some of the bacteria will take up the DA fragment alone, others will take up the vector lacking the DNA fragment and others will take up the vector containing the inserted fragment (see below). Indicate under each figure, whether the bacterial cell in question can tolerate antibiotic and whether it has green fluorescence.



1. *tolerates antibiotic since resistance gene is present*
2. *tolerates antibiotic since resistance gene is present and has green fluorescence because GFP can be expressed from the expression vector*
3. *Cannot grow in the presence of antibiotic since the resistance gene is absent. Doesn't have green fluorescence, as the gene encoding GFP can't be expressed in the bacterium unless it is inserted into an expression vector.*

You wish to isolate bacteria belonging to each of these three groups. Describe a strategy (which screening or selection will you use?) to isolate (1) bacteria containing the plasmid without the insert (2) bacteria containing the plasmid with inserted fragment (3) bacteria not containing any plasmid

One possible strategy could be:

The bacterial culture containing all groups is plated on a medium where all can survive and form colonies. This plate is replicated onto a medium containing antibiotic. Only those bacteria containing the plasmid (groups 1 and 2) can survive. By comparing this plate with the first plate, group 3 colonies can be identified (grow on plate 1, but not on plate 2). By screening plate 2 under UV light, we can distinguish group 2 (green fluorescence) from group 1 (no fluorescence).

(p. 390-391)